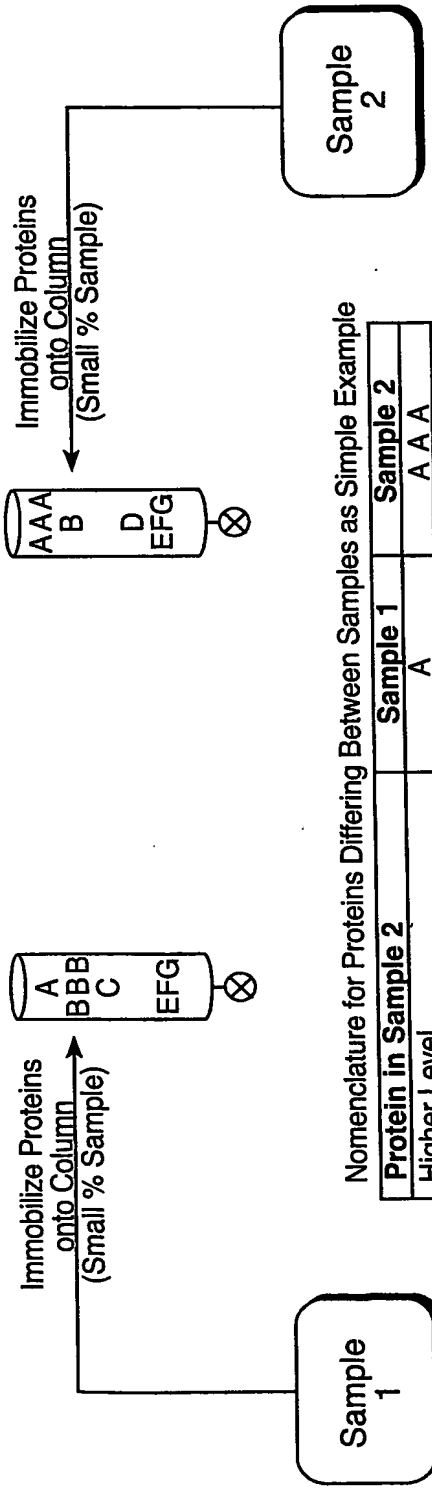
FIG. 1

Preparation of Protein Affinity Matrices



Nomenclature for Proteins Differing Between Samples as Simple Example

Protein in Sample 2	Sample 1	Sample 2
Higher Level	A	A A A
Lower Level	B B B	B
Not Pesent	C	None
Novel Species	None	D
Same Level	E F G	E F G
Not Present in Either Sample	H I J Absent	H I J Absent

Upper Case Letter is Protein, eg "A"

Lower Case Letter is Phage which binds to corresponding protein, eg "a"

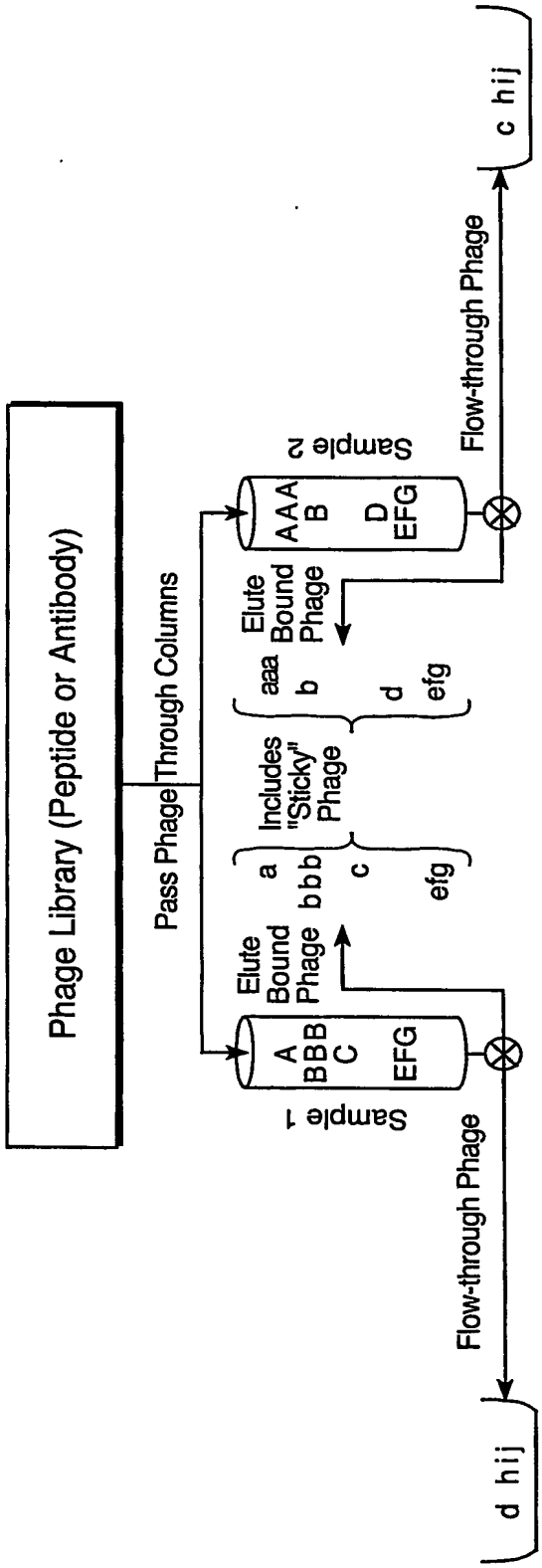

Capture Step One
Phage Library (Peptide or Antibody)
Pass Phage Through Columns
Sample 1
A BBB C EFG
Sample 2
AAA B D EFG
Elute Bound Phage
Includes "Sticky" Phage
a bbb c efg
aaa b d efg
Flow-through Phage
Flow-through Phage
c hij
d hij

FIG. 2

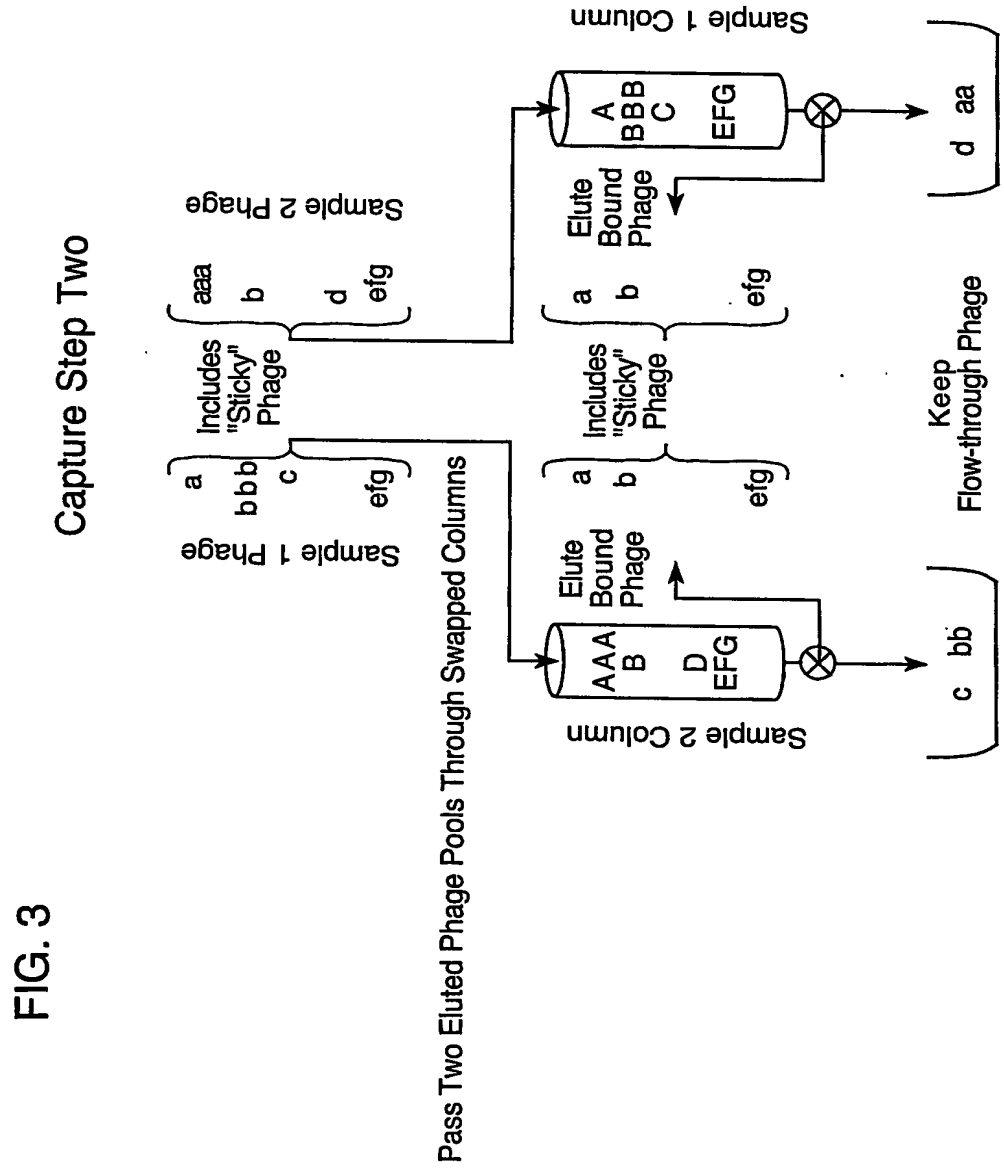

Capture Step Two
Sample 1 Phage
a
b b b
c
efg
Includes "Sticky" Phage
Sample 2 Phage
aaa
b
d
efg
Includes "Sticky" Phage
Pass Two Eluted Phage Pools Through Swapped Columns
Sample 2 Column
A A A
B
D
E F G
Elute Bound Phage
a
b
efg
Keep Flow-through Phage
c bb
Sample 1 Column
A
B B B
C
E F G
Elute Bound Phage
a
b
efg
Keep Flow-through Phage
d aa

FIG. 3

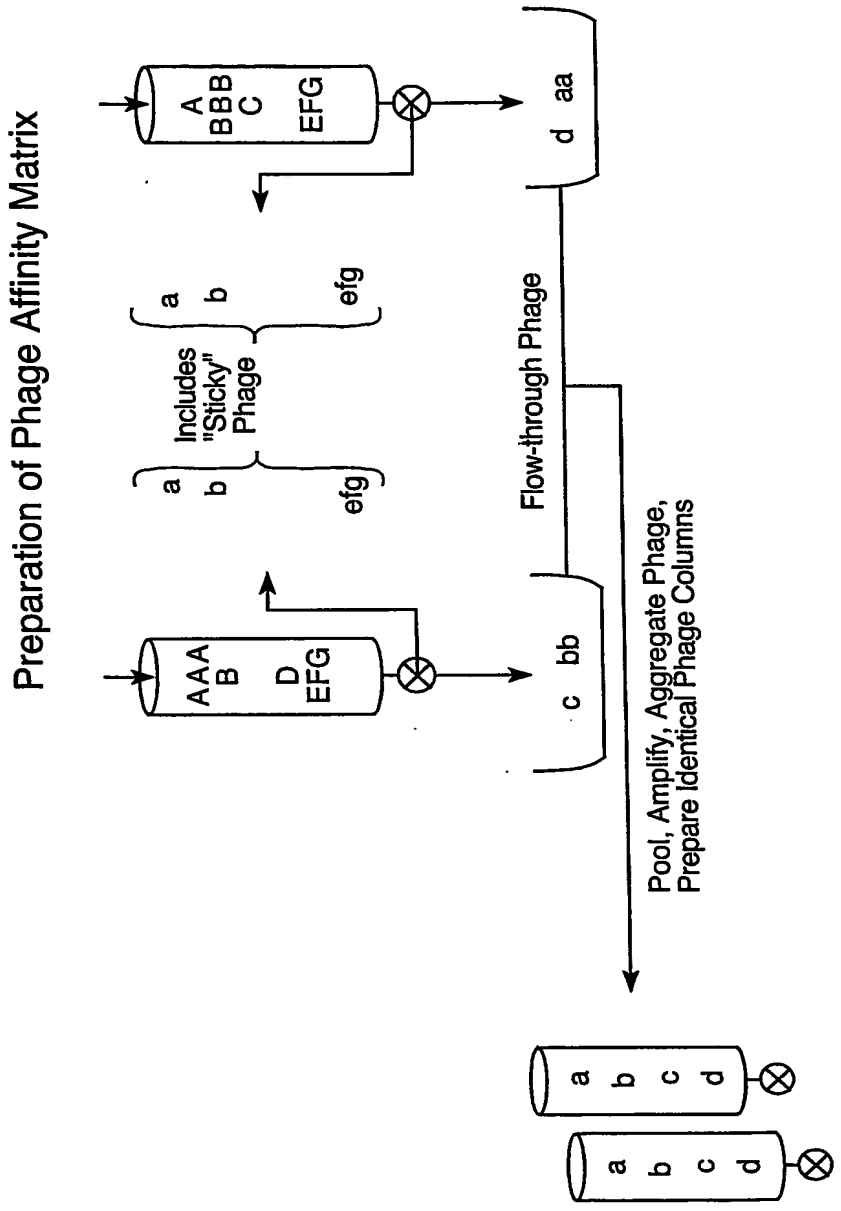
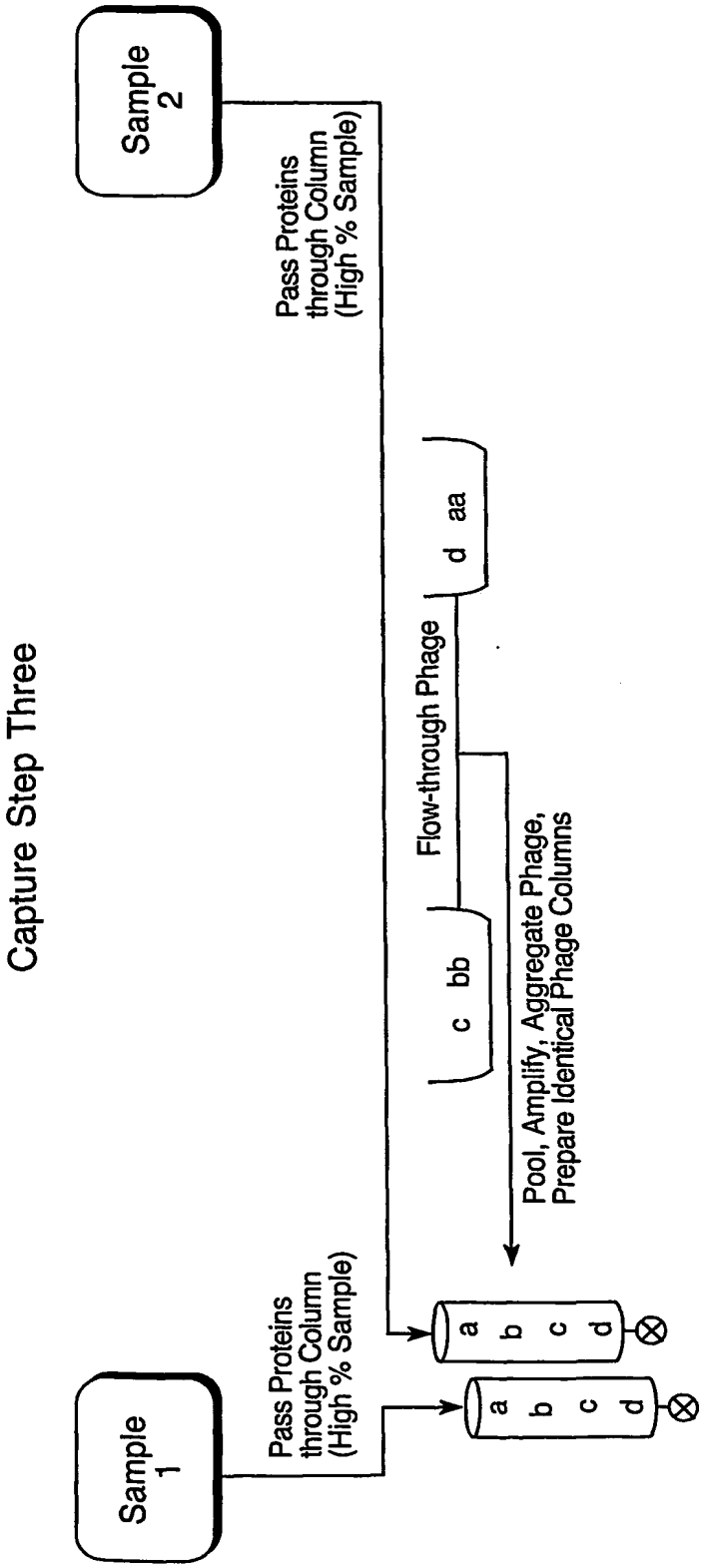

Preparation of Phage Affinity Matrix
A
BBB
C
EFG
d aa
a
b
efg
Includes
"Sticky"
Phage
a
b
efg
Flow-through Phage
AAA
B
D
EFG
c bb
Pool, Amplify, Aggregate Phage,
Prepare Identical Phage Columns
a b c d
a b c d

FIG. 4

FIG. 5


Capture Step Three
Sample 1
Sample 2
Pass Proteins through Column (High % Sample)
Pass Proteins through Column (High % Sample)
a b c d
a b c d
Flow-through Phage
c bb
d aa
Pool, Amplify, Aggregate Phage, Prepare Identical Phage Columns

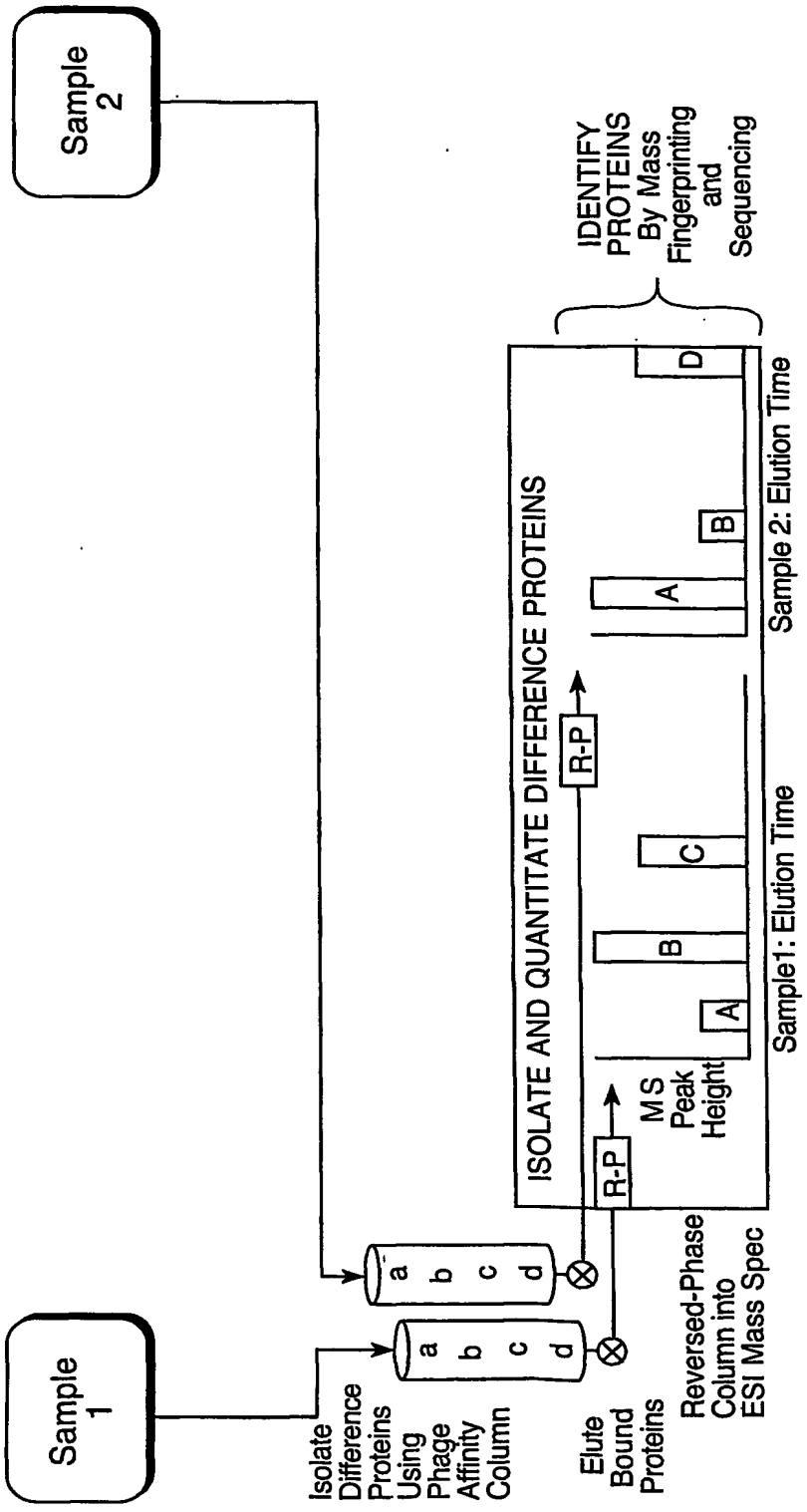

Quantitation and Identification of Difference Proteins
Sample 1
Sample 2
Isolate Difference Proteins Using Phage Affinity Column
a b c d
Elute Bound Proteins
R-P
Reversed-Phase Column into ESI Mass Spec
ISOLATE AND QUANTITATE DIFFERENCE PROTEINS
MS Peak Height
A
B
C
D
Sample1: Elution Time
Sample 2: Elution Time
IDENTIFY PROTEINS By Mass Fingerprinting and Sequencing

FIG. 6

FIG. 7

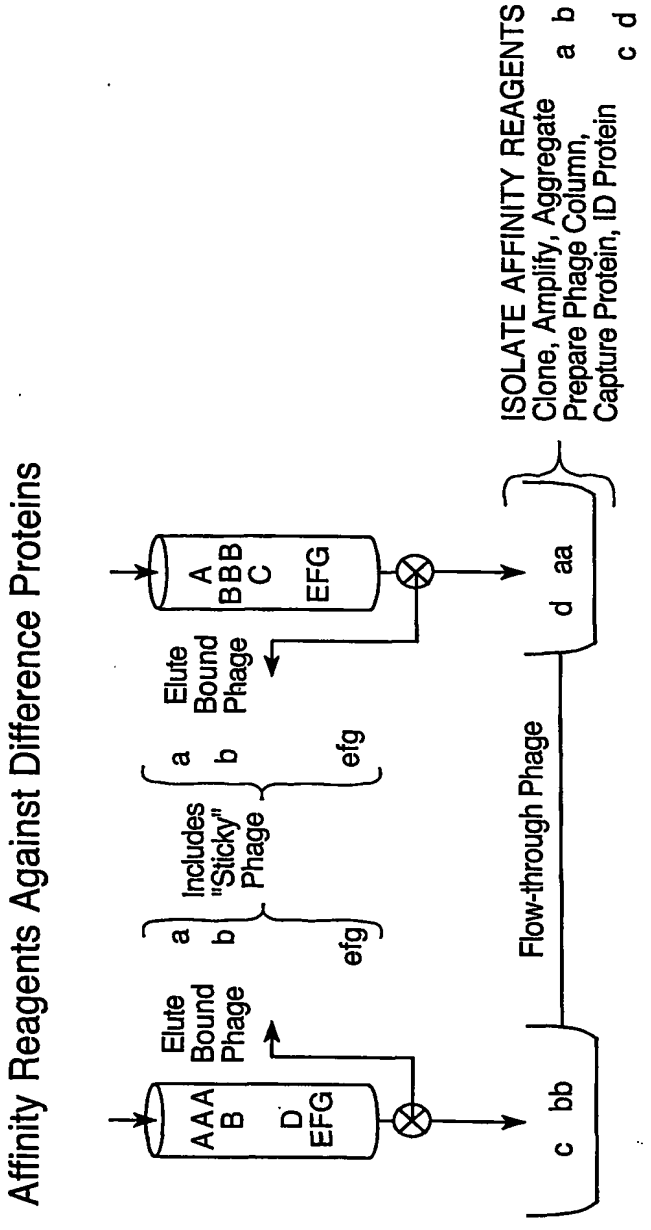

Affinity Reagents Against Difference Proteins
AAA
B
D
EFG
Elute
Bound
Phage
a
b
efg
Includes
"Sticky"
Phage
A
BBB
C
EFG
Elute
Bound
Phage
a
b
efg
c bb
Flow-through Phage
d aa
ISOLATE AFFINITY REAGENTS
Clone, Amplify, Aggregate a b
Prepare Phage Column,
Capture Protein, ID Protein c d

FIG. 8

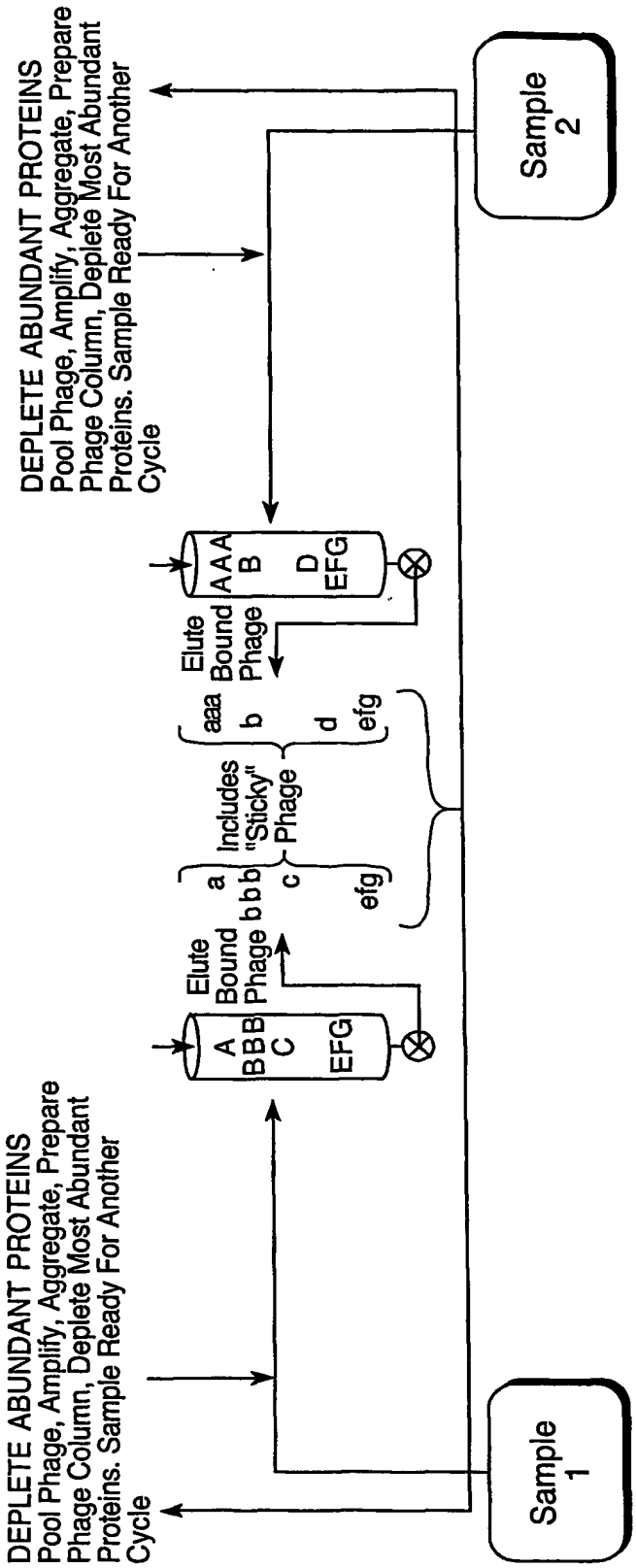
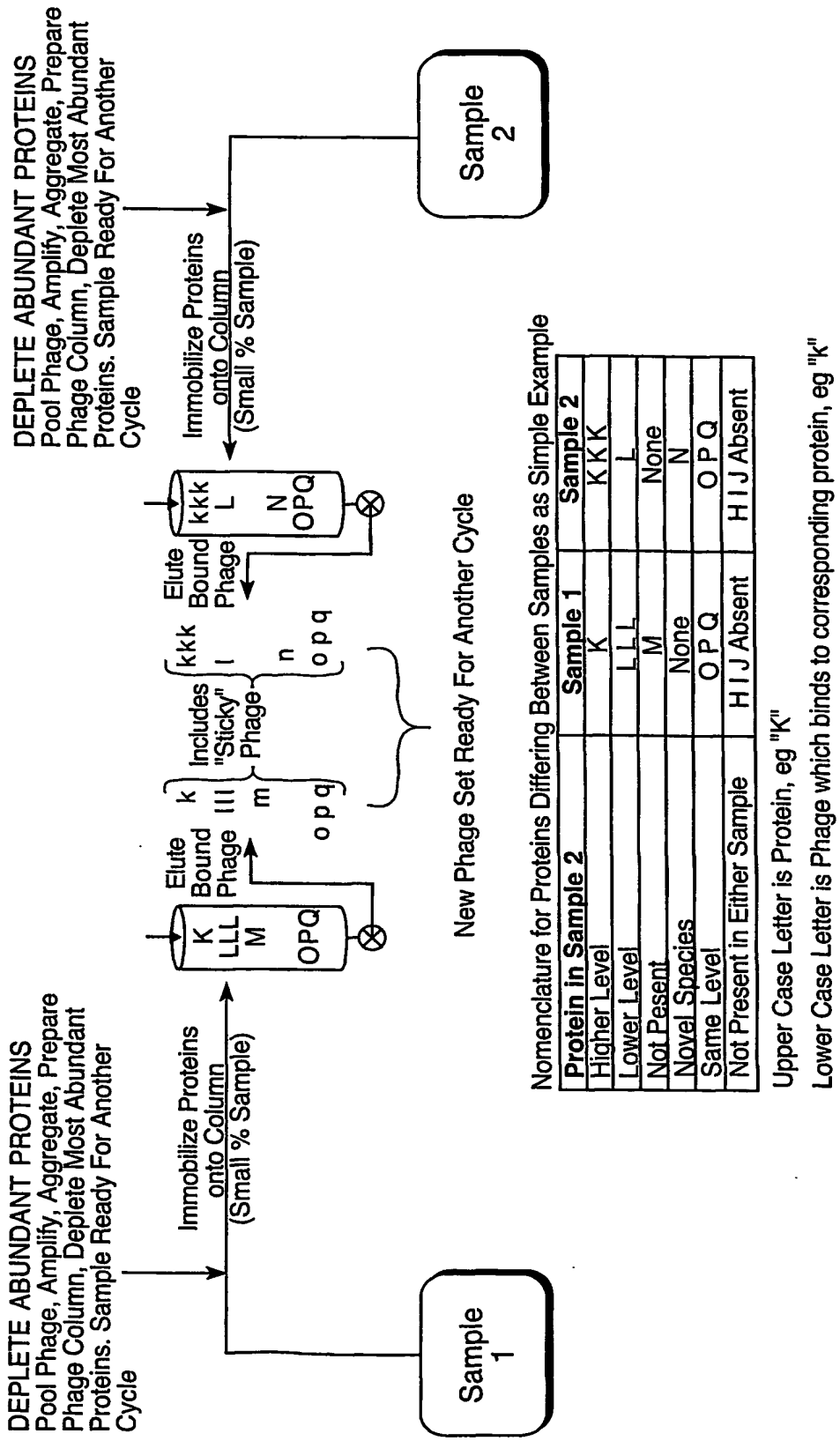

Depletion of Most Abundant Proteins
DEPLETE ABUNDANT PROTEINS
Pool Phage, Amplify, Aggregate, Prepare
Phage Column, Deplete Most Abundant
Proteins. Sample Ready For Another
Cycle
Sample 1
A
BBB
C
EFG
Elute
Bound
Phage
b b b
a
c
Includes
"Sticky"
Phage
efg
aaa
b
d
efg
Elute
Bound
Phage
AAA
B
D
EFG
DEPLETE ABUNDANT PROTEINS
Pool Phage, Amplify, Aggregate, Prepare
Phage Column, Deplete Most Abundant
Proteins. Sample Ready For Another
Cycle
Sample 2

FIG. 9



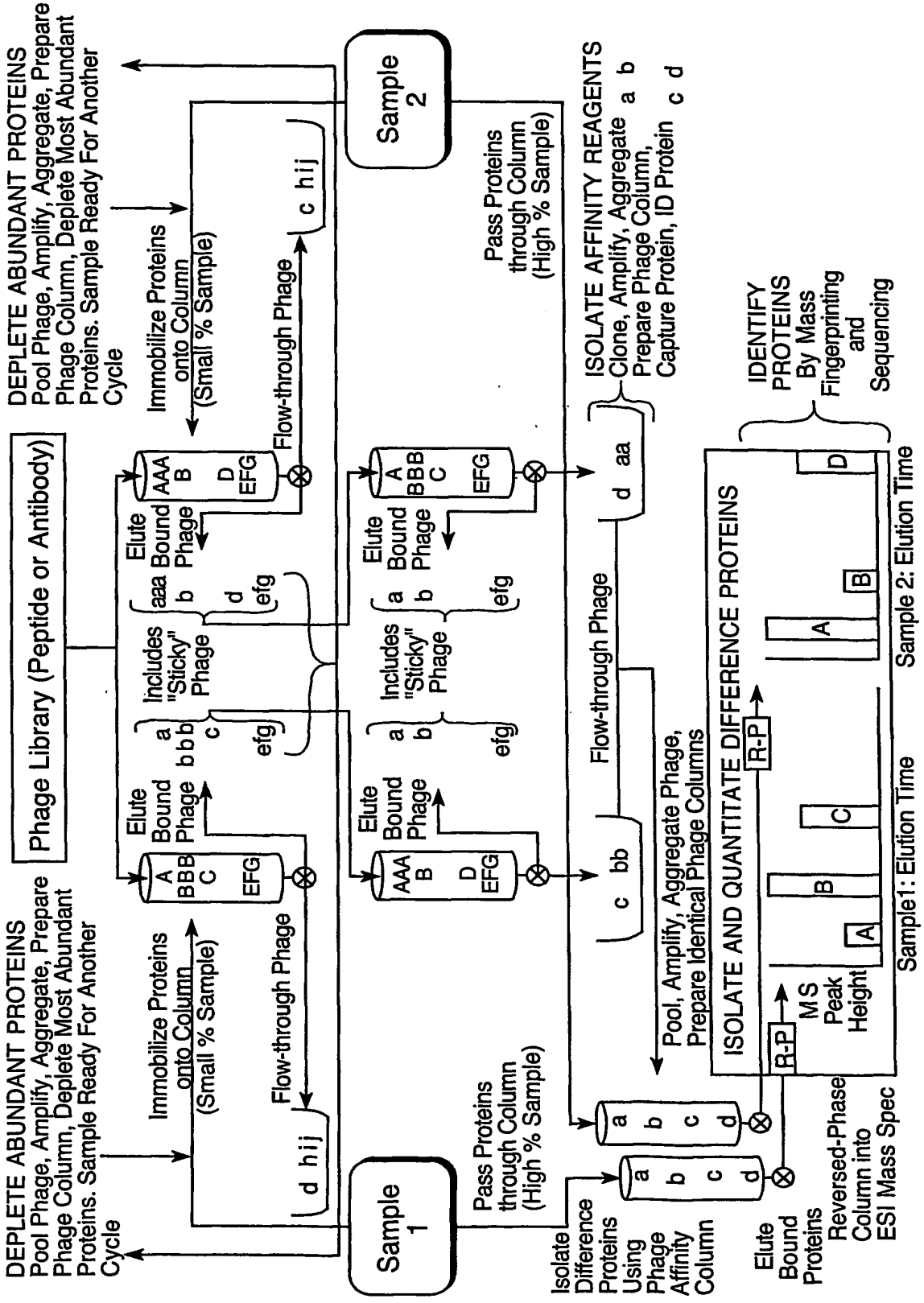
Nomenclature for Proteins Differing Between Samples as Simple Example

Protein in Sample 2	Sample 1	Sample 2
Higher Level	K	K K K
Lower Level	L L L	L
Not Pesent	M	None
Novel Species	None	N
Same Level	O P Q	O P Q
Not Present in Either Sample	H I J Absent	H I J Absent

Upper Case Letter is Protein, eg "K"

Lower Case Letter is Phage which binds to corresponding protein, eg "k"

FIG. 10 Differential Phage Capture Proteomics Summary



Example: Human Plasma

4 x 10^5 protein species (estimated), 12 orders of magnitude relative abundance*

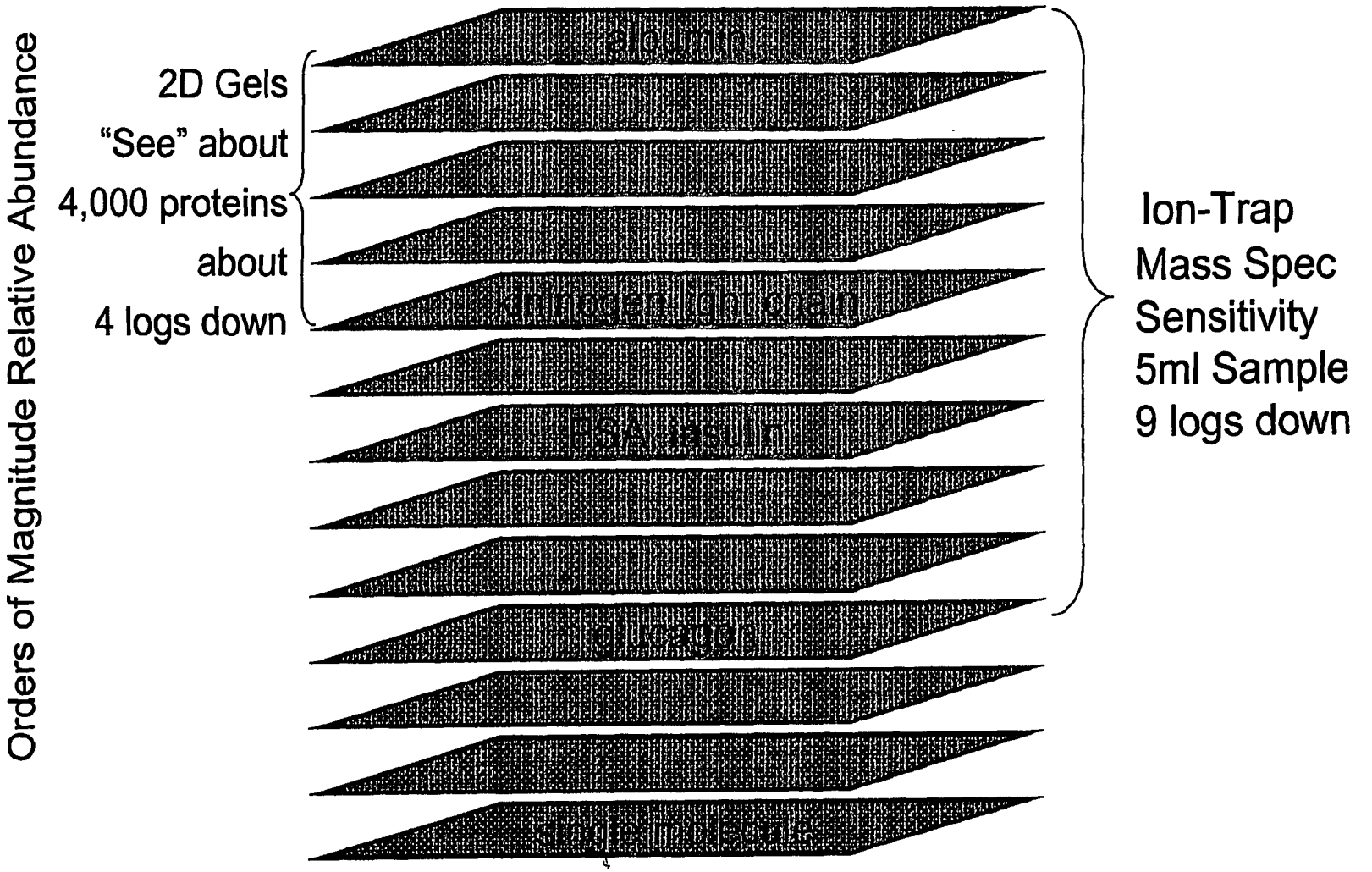


Figure 11

*Abundance data from Corthals, Wasinger, Hochstrasser and Sanchez; Electrophoresis **2000**, 21, 1104-1115

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10/518721

Protein Abundance Window Analysis

Optimize analysis through Phage-to-Protein ratio

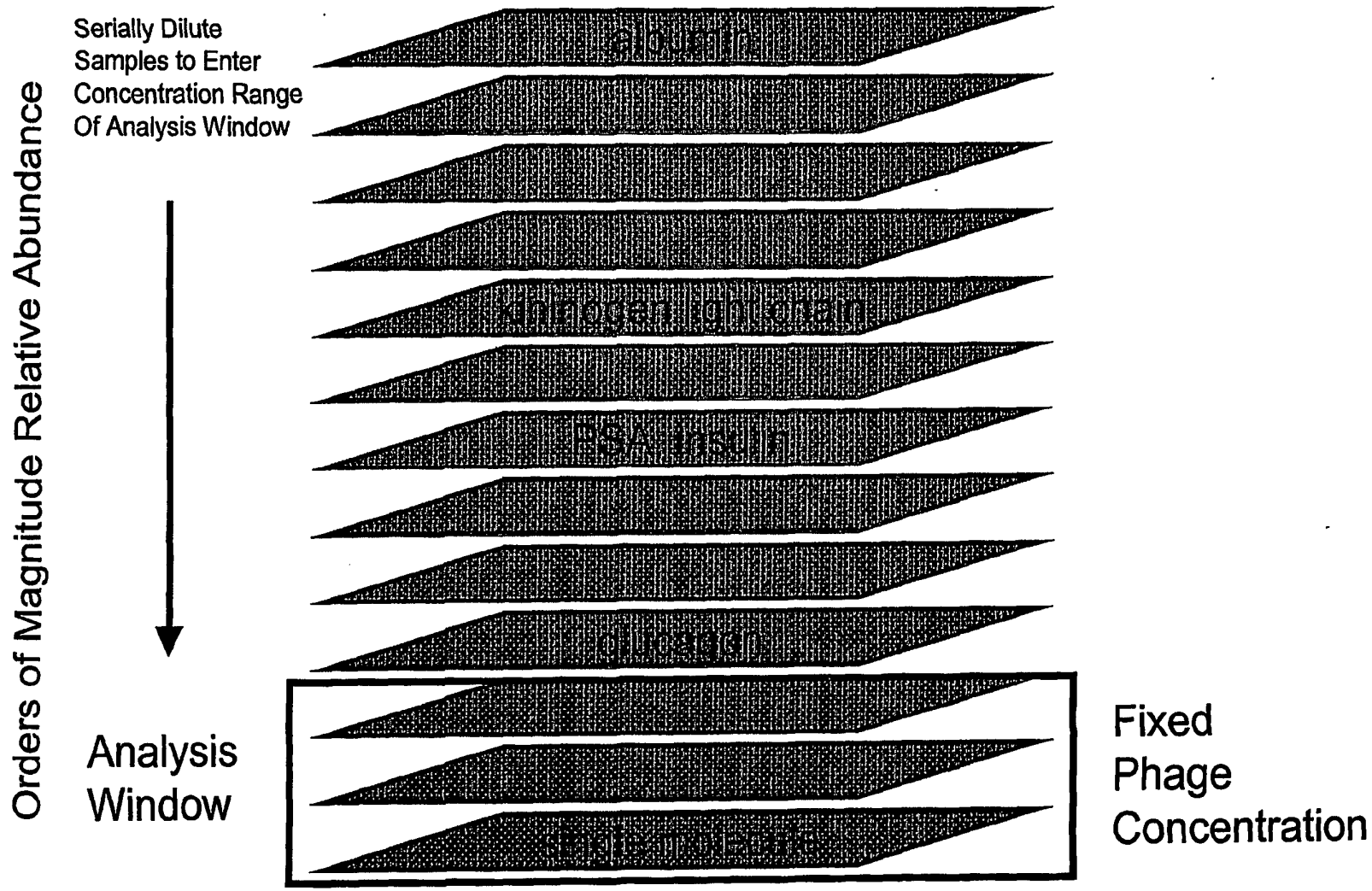

Figure 12

WO 2004/001377
PCT/US2003/019613

PROTEIN ABUNDANCE WINDOW ANALYSIS

REPRESENTATION OF PROTEIN SPECIES PRESENT IN TWO HUMAN PLASMA SAMPLES FOR COMPARISON

THE ISOLATION OF PHAGE AGAINST SPECIES CHANGING BETWEEN TWO SAMPLES

1,000,000,000,000 Z + 2,000,000,000,000 Z	1,000,000,000,000 Z + 20,000,000,000,000 Z	1,000,000,000,000 Z + 200,000,000,000,000,000 Z	1,000,000,000,000 Z + 1,000,000,000,000,000 Z
100,000,000,000 Z + 200,000,000,000 Z	100,000,000,000 Z + 2,000,000,000,000 Z	100,000,000,000 Z + 20,000,000,000,000,000 Z	100,000,000,000 Z + 100,000,000,000,000 Z
10,000,000,000 Z + 20,000,000,000 Z	10,000,000,000 Z + 200,000,000,000 Z	10,000,000,000 Z + 2,000,000,000,000,000 Z	10,000,000,000 Z + 10,000,000,000,000 Z
1,000,000,000 Z + 2,000,000,000 Z	1,000,000,000 Z + 20,000,000,000 Z	1,000,000,000 Z + 200,000,000,000,000 Z	1,000,000,000 Z + 1,000,000,000,000 Z
100,000,000 Z + 200,000,000 Z	100,000,000 Z + 2,000,000,000 Z	100,000,000 Z + 20,000,000,000,000 Z	100,000,000 Z + 100,000,000,000 Z
10,000,000 Z + 20,000,000 Z	10,000,000 Z + 200,000,000 Z	10,000,000 Z + 2,000,000,000,000 Z	10,000,000 Z + 10,000,000,000 Z
1,000,000 Z + 2,000,000 Z	1,000,000 Z + 20,000,000 Z	1,000,000 Z + 200,000,000,000 Z	1,000,000 Z + 1,000,000,000 Z
100,000 Z + 200,000 Z	100,000 Z + 2,000,000 Z	100,000 Z + 20,0000,000 Z	100,000 Z + 100,000,000 Z
10,000 Z + 20,000 Z	10,000 Z + 200,000 Z	10,000 Z + 2,000,000 Z	10,000 Z + 10,000,000 Z
1,000 Z + 2,000 Z	1,000 Z + 20,000 Z	1,000 Z + 200,000 Z	1,000 Z + 1,000,000 Z
100 Z + 200 Z, Δ=z	100 Z + 2,000 Z, Δ=z	100 Z + 20,000 Z, Δ=z	100 Z + 100,000 Z, Δ=z
10 Z + 20 Z, Δ=10z	10 Z + 200 Z, Δ=91z	10 Z + 2,000 Z, Δ=91z	10 Z + 10,000 Z, Δ=91z
Z + 2 Z, Δ=z	Z + 20 Z, Δ=19z	Z + 200 Z, Δ=100z	Z + 1,000 Z, Δ=100z

General Protein Species named "Z"

All phage that bind to Protein Species Z named "z"

Proportions of Protein Species in Sample 1 compared to Sample 2 represented as: $n_1Z + n_2Z$

Number of phage remaining after column swap step represented as: $\Delta = n_3z$

In this example, 101 phage particles are used

Therefore $\Delta = 101 - n_1z$

Pass fixed amount of phage through serially diluted immobilized proteins
Pool all phage captured

The shaded area indicates the successful generation of phage against species changing between the two samples
This results from the appropriate ratio of numbers of phage to numbers of proteins

Figure 13

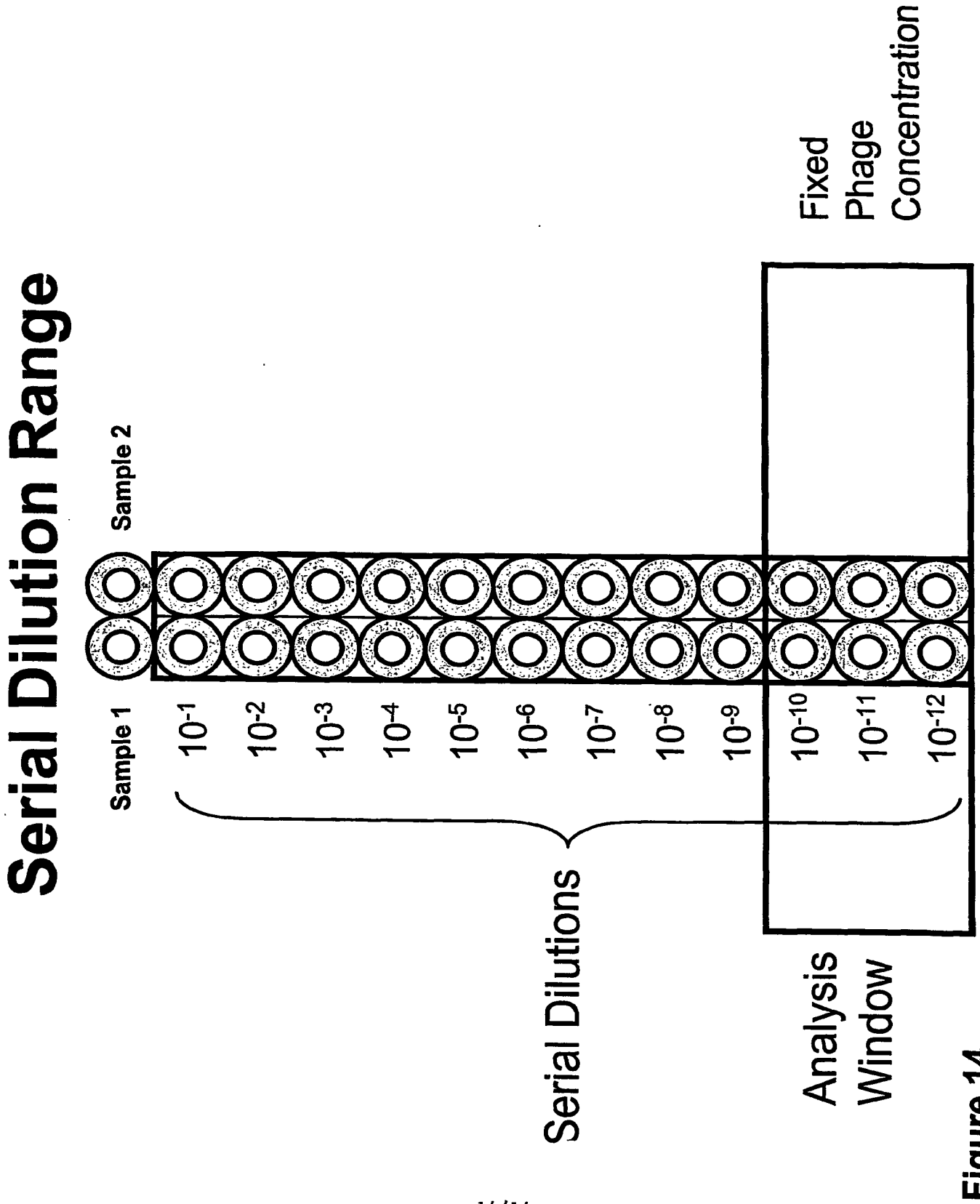
Serial Dilution Range
Sample 2
Sample 1
10-1
10-2
10-3
10-4
10-5
10-6
10-7
10-8
10-9
10-10
10-11
10-12
Serial Dilutions
Analysis Window
Fixed Phage Concentration

Figure 14